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METHOD FOR THE PRODUCTION OF OILS WITH HIGH OXIDATIVE STABILITY FROM MARINE RAW MATERIAL

[Fremgangsmåte for produksjon av oljer med høy oksidativ stabilitet fra marint råstoff]

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TITLE (54): METHOD FOR THE PRODUCTION OF OILS WITH HIGH OXIDATIVE STABILITY FROM MARINE RAW MATERIAL

FOREIGN TITLE (54A): FREMGANGSMÅTE FOR PRODUKSJON AV OLJER MED HØY OKSIDATIV STABILITET FRA MARINT RÅSTOFF

This invention concerns a method for the production of oils /2* with high oxidative stability from marine raw material.

Marine fat is unique in the sense that it contains large quantities of unsaturated fatty acids, but it is precisely the quantities of unsaturated (especially multiply unsaturated) fatty acids that expose marine fat to oxidation. Multiply unsaturated fatty acids oxidize more rapidly than monounsaturated fatty acids, stated simply, because more attachment points are present in the molecule. Low-temperature processes are therefore especially suitable for raw material rich in unsaturated fat.

The interest in marine fat has recently increased since Dyerberg and fellow workers suggested that the low mortality rate from diseases of the heart (Coronary Heart Disease, CHD) among the Eskimos of Greenland could be attributed to their marine diet[1]. The interest has been concentrated in particular on those multiply unsaturated fatty acids[2,3], which are especially abundant and which have unique positioning of the double bonds in the marine material. The most recent focus on multiply unsaturated fat that is the cause of low mortality from diseases of the heart has led to great commercial interest in products with their origin in marine fat. One of the greatest problems confronting these industries is the stabilization of this fat. Vitamin E (dl-a-tocopherol) is at present widely utilized as a stabilizer for these products, but oxidation still seems to be a problem.

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*Numbers in the margin indicate pagination in the foreign text.

Methods are in use today for the stabilization of marine fat for utilization in foodstuffs, such as margarine, sausages, salad dressings, etc.[4-8] Various strategies such as: improved refinement processes[7], the application of absorbent agents, the development of emulsions with a high content of marine fat[9-16], microencapsulation[10,11] and the application of various antioxidants[12-15] are being tested.

THE EFFECTS OF HEATING DURING THE TRADITIONAL EXTRACTION OF MARINE OILS

The traditional extraction of cod-liver oil

Present-day methods for the extraction of cod-liver oil involve the heating of cod livers, usually with the aid of water steam, until the cellular structure is destroyed and the proteins denatured. The result of this "steaming" of the raw material is that the cod-liver oil, which is insoluble in water, floats to the surface and can therefore be separated by centrifuging, pressing, flotation or ~~decanting~~.

The traditional extraction of fish oils

Fish oils are produced as a by-product of fish-meal production. This is also reflected by the quality of the oil. The process for the production of raw oil starts with the wet-pressing of the raw material. The raw material is heat-treated, filtered and the fat centrifuged/separated from the aqueous phase. The present-day herring-meal industry employs heating to between 70 and 90°C in order to denature the proteins and liberate the fat from the same. Alfa-Laval has, by developing the Condec process[17], reduced that time

during which the raw material is exposed to high temperature and thus a certain increase in quality.

The effects of heat treatment

Heat treatment causes, in addition to the denaturing of proteins, the decomposition of thermally labile antioxidants, the liberation of pro-oxidants, inactivation of enzymes that protect against oxidation and the thermal activation of oxidation reactions.

Thermal oxidation

High processing temperatures result both in the supplying of large quantities of energy for activation and higher reaction rates in the case of the oxidation reactions (auto-oxidation / photo-oxidation / enzymatic oxidation / polymerization).

The thermal decomposition of antioxidants

Heat treatment causes the activation of oxidation reactions that are either purely thermal or resulting from reduction of the content of (thermolabile) antioxidants. Heating leads to the inactivation of natural substances that contribute to making the fat stable against rancidity.

The solubility of odorizing, flavoring and coloring substances

Components that provide flavor, odor or color in the product are highly soluble in fat at high temperature.

Oil extracted by this method must therefore be submitted to extensive filtering processes to remove unwanted odorizing and flavoring substances (refinement) and, since natural antioxidants have been destroyed during extraction, needs to be stabilized against turning rancid with the addition of antioxidant. → add antioxidant

The denaturing of proteins /4

Enzymes that protect the lipids against oxidation (catalase glutathione peroxidase, superoxide dismutase, etc.) are inactivated by heat treatment. Metallic ions are liberated during the denaturing of proteins by heat treatment. Examples are ferritin and hemoglobin, which liberate iron during denaturing. Ions of transition metals like iron (Fe^{+3}) and copper (Cu^{2+}) are examples of strong pro-oxidants that contribute to the more rapid oxidation of the fat and, by that means, to lower stability.

Oil extraction with the aid of cold pressing

Techniques were developed for "cold pressing" (30-50°C) fat from organic material. This is an alternative method for extraction using organic solvents during the production of vegetable oils. The combination of mechanical processing and a moderate increase in temperature are utilized to drive out the fat. "Cold pressing" has also been applied to the production of cod-liver oil, but the fat, aside from moderate heating, is often exposed there to heating to 90°C for more effective separation in later processing steps.

OXIDATION

The oxidation of fat causes a deterioration of the taste and odor of those products where it takes place. Biologically active components are also lost in the case of rancidity, and that yields rancidity products (peroxides and radicals) that can be harmful to health.

The oxidation of unsaturated fatty acids is not a single reaction, but a complex system with several reactions having different ways of reacting and reaction products (auto-oxidation / photo-

oxidation / enzymatic oxidation). Generally, hydrogen is removed from double bonds in unsaturated fatty acids to produce free radicals that, in combination with oxygen, yield peroxide radicals. These will react again with the fat and produce hydroperoxide, which is the primary intermediate product in auto-oxidation processes. The peroxides and peroxide radicals are reactive molecules and can either decompose into smaller molecules such as aldehydes, hydrocarbons, esters, acids and alcohols, or oxidize further to yield dihydroperoxides, epoxy-hydroperoxides and cyclical endoperoxides. In the case of another central way of reacting, there is a combination of free radicals that yields dimers and polymers.

THE INVENTION /5

The goal of the task at hand is to stabilize the product, that is to say, expose it to the least possible oxidative stress in the case of production. This can be best accomplished as follows: brief transition time from raw material to product, avoidance of air (oxygen), the use of vacuum and inert gas, avoidance of contact with iron and copper (very strong pro-oxidants), avoidance of light and temperatures kept low during the entire process.

The consequences of freezing are focused on repackaging the membrane lipids. For the fat deposit, which consists mainly of triglycerides, freezing has no other consequences worth mentioning than a volume decrease in the fat vacuoles by about 10%. For membrane lipids, on the other hand, repackaging can directly damage membrane structures. In membranes, which consist in their original form of a flowing heterogeneous phospholipid structure, freezing gives rise to phase separation, the phospholipids being separated out as separate

"eyes" in the membrane. This phase separation is not reversible, and the alterations affect the function of the membrane after thawing.

In the frozen state, water, fat and tissue (cells) form a solid, rigid structure. This rigid structure comprising ice crystals, cell membrane and fat vacuoles is utilized for the mechanical processing of the frozen tissue. This effect is so great that it is not needed with gentle freezing. The elasticity in cell membranes has disappeared in the frozen state, and there is no possibility, when in the frozen state, of distributing the internal force that is exerted upon the tissue. This is enough to crush the tissue so that the oil can be extracted with moderate heating. For the best results of the invention, the freezing speeds will thus be high in order to obtain oil of good quality. With this method, the processing times are shortened, the oils being exposed for the shortest time possible to attack by those pro-oxidative substances present in the liver.

With gentle freezing, kernels are formed and, as a result, large ice crystals. The salt concentration in the cells is thereafter so high that it will itself have pro-oxidative properties. An increase in enzymatic activity is seen in the temperature range for ice formation (-2 - 0°C). This is an argument for the need to find the most rapid freezing methods possible for the production of stable, high-quality oils.

In the case of the present invention, the raw material is /6 material is not exposed to temperature conditions in which undesirable flavoring and coloring substances become soluble in the fatty phases or which destroy natural antioxidants.

This is achieved by the fact that the raw material is first of all frozen rapidly, so that the water in the material will form many small ice crystals, and the cell membranes and other cellular components will remain a firm, rigid structure.

During the subsequent mechanical processing of the raw material, for example, grinding, the fatty phases can be individually separated from the proteinaceous/aqueous phases after moderate warming. The fat melts out and can then be separated from the residues by pressing, flotation, decanting or centrifuging.

Oxidative stability is increased with the use of a low processing temperature, because the latter protects the natural antioxidants and requires less energy for activation and, as a result, contributes to lower reaction rates for the rancidity reactions. At low temperature, the fat also has low solubility for products that produce taste, smell and color in the product.

Fat that is highly unsaturated has a low melting point. The method is therefore particularly suitable for raw material rich in unsaturated fat.

The oxidation of oils causes deterioration of the taste and odor of products in which it takes place. Biologically active components are also lost in the case of rancidity, and that yields rancidity products (peroxides and radicals) that can be harmful to health.

In the case of traditional extraction in which vigorous heating of the raw material is employed for fat separation, the residual material (waste) consists among other things of denatured proteins, including denatured connective tissue. This waste has at the same time poor food value as a protein source, because it is difficult to

digest. An alternative method for the extraction of fat at low temperature yields a waste with far greater nutritional value. In the case of low-temperature processes, the proteins are not denatured, and the oxidation of lipids is inhibited. This waste is therefore a good source of both phospholipids and proteins.

Purpose

The goal of the present invention is to make a method available for the stabilization of fat, preventing this fat from oxidizing during extraction and to make it stable for packaging, storage, distribution and sale.

Another purpose is to produce a method for the production of a /7
stable marine oil, which at the same time prevents odorant and flavoring substances from being extracted in the fact from the marine raw material.

Furthermore, one goal is to produce a procedure for the production of fat, which yields more usable by-products and is therefore environmentally friendly.

The method is characterized by the following advantages:

1. The fat extracted has high oxidative stability, is nearly colorless, has a mild and neutral taste and a higher degree of unsaturation.
2. Lower fat temperature during extraction.
    a. No antioxidants are denatured.
    b. Lower solubility of odorant and flavoring substances in the fat.
    c. Pro-oxidants are not liberated.
    d. Lower degree of activating chemical reactions.

e. Lower reaction rate in oxidation reactions.

3. High yield of fat from extraction.

a. Effective bursting of cell membranes is achieved.

b. Coagulation of proteins with the subsequent encapsulation of fat being avoided.

4. The use of organic solvents is avoided.

Examples: /8

Example 1

180 g of liver from the cod (*Gadus morhua*) were chilled and then frozen at a core temperature of -6°C. The liver was then homogenized in the frozen condition. The fat liberated from the raw material was heated to 6°C, and water and protein were separated by centrifuging the mass for 5 minutes at 3,000 rpm. The fat yield from the use of this method was 84%. The fat extracted has high oxidative stability; the peroxide value (PV) = 0.5 meq, the anisidine value (AV) = 0. The product is nearly colorless, has a mild and neutral taste and a higher degree of unsaturation than was the case with traditional methods of extraction.

For comparison, 180 g of cod liver were extracted with the use of the traditional extraction process, employing the direct application of steam. The fat yield from the use of this method was 81%. The fat extracted had a peroxide value (PV) = 2 meq and anisidine value = 11. The product was yellow in color, has a typical cod-liver oil taste and a lower degree of unsaturation than that from the new process.

Example 2

Muscle from fresh salmon (*Salmo salar*) was frozen at -6°C and then homogenized in the frozen state. The fat was liberated from the

raw material by heating it to 10°C. The fat was separated from water and protein by centrifuging the mass for 10 minutes at 3,000 rpm. The peroxide value (PV) and anisidine value (AV) were determined at PV = 0.5 meq and AV = 0.

For comparison, fat from salmon muscle was extracted by the use of a nonindustrial method (Bligh and Dyer, 1959)[18]. The fat extracted had a peroxide value (PV) ≈ 1 meq and an anisidine value (AV) ≈ 0.

Example 3 /9

Muscle from a completely frozen mackerel (*Scomber scombrus*) was purchased from a local grocery store (completely frozen, core temperature -10°C) and homogenized with the aid of a grinder. The fat was liberated when the raw material was heated to 10°C. Fat, water and protein were separated by centrifuging the mass for 10 minutes at 3,000 rpm. The peroxide value (PV) and anisidine value (AV) were determined at PV = 7 and AV = 1.2. For comparison, fat from the purchased mackerel was extracted by the use of a nonindustrial method (Bligh and Dyer, 1959)[18]. The fat extracted had a peroxide value (PV) ≈ 35 meq and an anisidine value (AV) ≈ 7.

Patent claims /10

1. Method for the production of oils with high oxidative stability from marine biological material, **characterized by the fact** that the raw material is rapidly frozen and crushed in the frozen state, and the fat is separated by low heating until the fat is fluid.
2. Method for the production of oils with high oxidative stability according to Claim 1, **characterized by the fact** that the marine raw material is preferably frozen rapidly, so that many small ice